

APPENDIX B

Claims as Pending Following Entry of Amendment

1. A recombinant thermostable DNA polymerase which is characterized in that
 - a) in its native form said polymerase comprises the amino acid sequence
LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4, 6, 9, and 10 of said sequence are any amino acid residue, and "Xaa" at position 7 of said sequence is Val or Ile;
 - b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu; and
 - c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.
2. The recombinant thermostable DNA polymerase of claim 1 wherein said nucleotide is a dideoxynucleotide and said level of discrimination is at least 3-fold lower than that of said native form of said polymerase.
3. The recombinant thermostable DNA polymerase of claim 2 wherein said level of discrimination is measured by determining the concentration of a dideoxynucleotide labeled with a fluorescein dye that is required for 50% inhibition of DNA synthesis.
4. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said polymerase is from a thermophilic species selected from the group consisting of *Thermosiphon africanus*, *Bacillus caldotenax*, and *Bacillus stearothermophilus*.

5. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said polymerase is from a *Thermus* species.

6. (Amended) The recombinant thermostable DNA polymerase of claim 5 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

7. (Amended) The recombinant thermostable DNA polymerase of claim 5 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

8. (Amended) The recombinant thermostable DNA polymerase of claim 7 wherein said "Xaa" at position 4 is mutated to Lys.

9. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said

polymerase is from a *Thermotoga* species.

10. (Amended) The recombinant thermostable DNA polymerase of claim 9 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

11. A nucleic acid sequence encoding a recombinant thermostable DNA polymerase which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4, 6, 9, and 10 of said sequence are any amino acid residue, and "Xaa" at position 7 of said sequence is Val or Ile;

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu; and

c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.

12. The nucleic acid sequence of claim 11 wherein said nucleotide is a dideoxynucleotide and said level of discrimination is at least 3-fold lower than that of said native form of said

polymerase.

13. The nucleic acid sequence of claim 12 wherein said level of discrimination is measured by determining the concentration of a dideoxynucleotide labeled with a fluorescein dye that is required for 50% inhibition of DNA synthesis.

14. The nucleic acid sequence of claim 12 wherein said polymerase is from a thermophilic species selected from the group consisting of *Thermosiphon africanus*, *Bacillus caldovenax*, and *Bacillus stearothermophilus*.

15. The nucleic acid sequence of claim 12 wherein said polymerase is from a *Thermus* species.

16. (Amended) The nucleic acid sequence of claim 15 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

17. (Amended) The nucleic acid sequence of claim 15 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence

LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

18. (Amended) The nucleic acid sequence of claim 17 wherein said "Xaa" at position 4 is mutated to Lys.

19. The nucleic acid sequence of claim 12 wherein said polymerase is from a *Thermotoga* species.

20. (Amended) The nucleic acid sequence of claim 19 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu.

21. (Amended) A method of DNA sequencing which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

- b) providing a dye-terminator labeled with a negatively charged fluorescent dye; and
- c) performing a dye-terminator sequencing reaction.

22. The method of claim 21 wherein said nucleotide is a dideoxynucleotide and said level of discrimination is measured by determining the ratio of the concentration of a dideoxynucleotide labeled with a fluorescein dye required for 50% inhibition of DNA synthesis versus the concentration of an unlabeled dideoxynucleotide required for 50% inhibition.

23. The method of claim 22 wherein said ratio is 4 or less.

24. The method of claim 22 wherein said polymerase is from a thermophilic species selected from the group consisting of *Thermosipho africanus*, *Bacillus caldotenax*, and *Bacillus stearothermophilus*.

25. The method of claim 22 wherein said thermostable DNA polymerase is from a *Thermus* species.

26. (Amended) The method of claim 25 wherein said amino acid sequence comprises LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

27. The method of claim 26 wherein said "Xaa" at position 4 is Lys.
28. The method of claim 22 wherein said polymerase is from a *Thermotoga* species.
29. (Amended) The method of claim 28 wherein said amino acid sequence comprises LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.
30. The method of claim 29 wherein said "Xaa" at position 4 is Arg.
31. (Amended) A method of producing labeled DNA which comprises:
- a) providing a thermostable DNA polymerase characterized in that
 - i) said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and
 - ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;
 - b) providing a nucleotide labeled with a fluorescein family dye; and
 - c) performing a DNA synthesis reaction.
32. (Amended) A method of producing labeled primer extension products which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile,

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase,

iii) said polymerase also comprises the second amino acid sequence

SQIXLR(V/I) (SEQ ID NO: 18) where "X" is any amino acid except E,

iv) said polymerase has a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

b) providing a ribonucleotide labeled with a fluorescein family dye; and

c) performing a primer extension reaction.

33. (Amended) A kit for DNA sequencing which comprises:

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase; and

b) a terminator labeled with negatively-charged fluorescent dye.

34. The kit of claim 33 wherein said reduced level of discrimination is measured by determining the ratio of the concentration of ddNTP labeled with a fluorescein family dye required for 50% inhibition of DNA synthesis compared to that for an unlabeled ddNTP and said ratio is 4 or less.

35. The kit of claim 34 wherein said amino acid sequence comprises:
LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

36. (Amended) The kit of claim 35 wherein said "Xaa" at position 4 is Lys.

37. (Amended) The kit of claim 34 wherein said amino acid sequence comprises
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

38. (Amended) The kit of claim 37 wherein said "Xaa" at position 4 is Arg.

39. (Amended) A kit for DNA sequencing which comprises:

a) a mutant thermostable DNA polymerase characterized in that

i) in its native form said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4,

6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 7 of this sequence is Val or Ile;

ii) said amino acid sequence is mutated, except that "Xaa" at position 4 is not mutated to Glu; and

iii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.

40. The kit of claim 39 wherein said level of discrimination is at least 5-fold lower than that of said native form of said polymerase.

41. The kit of claim 40 wherein said recombinant thermostable DNA polymerase is characterized in that in its native form said polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlalleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid.

42. (Amended) The kit of claim 41 wherein said "Xaa" at position 4 is mutated to Lys.

43. The kit of claim 40 wherein said recombinant thermostable DNA polymerase is characterized in that in its native form said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile.

44. (Amended) The kit of claim 43 wherein said "Xaa" at position 4 is Arg.

45. (Amended) A kit for producing labeled DNA which comprises:

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 7), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile,

ii) said polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase; and

b) a nucleotide labeled with a negatively-charged fluorescent dye.

46. (Amended) The kit of claim 45 wherein said amino acid sequence comprises

LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:14), whereby "Xaa" at position 4 is any amino acid except Glu.

47. (Amended) The kit of claim 45 wherein said "Xaa" at position 4 is Lys.

48. (Amended) The kit of claim 45 wherein said amino acid sequence comprises

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 15), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

49. (Amended) The kit of claim 48 wherein said "Xaa" at position 4 is Arg.

50. (Amended) A kit for producing labeled primer extension products which comprises:

a) a thermostable DNA polymerase which is characterized in that

i) in its native form, the polymerase comprises the first amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile;

ii) the polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

iii) the polymerase also comprises the second amino acid sequence SQIXLR(V/I) (SEQ ID No: 18) where "X" is any amino acid except E;

iv) the polymerase has a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase; and

b) a ribonucleotide labeled with a fluorescein family dye.

51. (Amended) The kit of claim 50 wherein said amino acid sequence comprises

LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

52. (Amended) The kit of claim 51 wherein said "Xaa" at position 4 is Lys.

53. (Amended) The kit of claim 50 wherein said amino acid sequence comprises

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

54. (Amended) The kit of claim 53 wherein said "Xaa" at position 4 is Arg.